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A Mechanistic Model of Effects of Dioxin on Thyroid Hormones in the Rat

MICHAEL C. KOHN,* CHARLES H. SEWALL,† GEORGE W. LUCIER,† AND CHRISTOPHER J. PORTIER*

*Laboratory of Quantitative and Computational Biology and †Laboratory of Biochemical Risk Analysis, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, North Carolina 27709

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A physiological dosimetric model of the disposition of TCDD in the rat (Kohn *et al., Toxicol. Appl. Pharmacol.* 120, 138–154, 1993) was extended to include effects of dioxin on serum concentrations of thyroid hormones in the rat. The extended model included distribution of blood among major vessels and tissue capillary beds and resorption of TCDD released into the gut lumen from the liver by cell lysis consequent to cytotoxicity. TCDD metabolism was represented by Hill kinetics. Parameter values were estimated by fitting time-course data for a single subcutaneous injection of TCDD and dose–response data for biweekly oral dosing. The extended model included new compartments for the thyroid and thyroxine-sensitive tissues (e.g., pituitary, kidney, and brown fat), secretion and tissue uptake of thyroid hormones, binding of 3,5,3′-triiodothyronine (T_3) and 3,5,3′,5′-tetraiodothyronine (thyroxine, T_4) to proteins in blood and tissues, deiodination of iodothyronines, and glucuronidation of T_4 by the hepatic UDP-glucuronosyltransferase (UGT) activity induced by TCDD. Secretion of thyroid hormones was modeled as regulated by thyrotropin (TSH), whose secretion was modeled as regulated by the hypothalamic factors thyrotropin releasing hormone and somatostatin. Release of the hypothalamic factors was modeled as under feedback control by the blood T_4 level. Induction of UGT was modeled as stimulated by the Ah receptor–TCDD complex. The extended model fit the observed dose–response of P450 isozymes and Ah and estrogen receptors following repeated oral doses with comparable accuracy as the earlier model. The fit to liver and fat TCDD levels following single and repeated oral and subcutaneous doses was improved over the earlier model. The revised model's predicted liver TCDD concentrations at very low doses were verified experimentally. The model reproduced the responses observed for blood T_3, T_4, and TSH after 31 weeks of biweekly oral dosing of rats with TCDD. The model also predicted responses of *UGT* mRNA and UGT enzymatic activity comparable to those observed in TCDD-treated rats in experiments whose data were not used in constructing the model. Calculated increases in blood TSH levels are consistent with prolonged stimulation of the thyroid and may represent an early stage in the induction of thyroid tumors identified in previous two-year bioassays. Thus, increases in UGT activity may be useful as a biomarker for tumorigenic changes in hormone levels subsequent to TCDD exposure. © 1996 Academic Press, Inc.

Exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) has been observed to cause tumors at several sites in both sexes of rats and mice (Huff *et al.*, 1991). Although TCDD's carcinogenic actions in female rat livers (Kociba *et al.*, 1978; National Toxicology Program, 1982) has been used to estimate human cancer risks from exposure to this chemical (Lucier *et al.*, 1993), the incidence of thyroid tumors in male rats and female mice (National Toxicology Program, 1982) may be a more sensitive end point. A quantitative dose–response relationship for a biomarker related to thyroid tumors might permit extrapolation of the incidence of this end point at doses used in laboratory experiments to the incidence predicted to result from environmental exposure.

Administration of a single oral dose of 25 μg TCDD/kg body weight to rats leads to reduced serum thyroxine (3,5,3′,5′-tetraiodothyronine, T_4) concentrations (Bastomsky, 1977; Gorski and Rozman, 1987; Gorski *et al.*, 1988). Treatment of rats with TCDD has been observed (Bastomsky, 1977) to increase the concentration of the serum glycoprotein thyrotropin (thyroid stimulating hormone, TSH). This result is consistent with the idea that the serum TSH level responds to changes in circulating T_4. TCDD treatment has been observed to have little effect on serum 3,5,3′-triiodothyronine (T_3) in rats (Bastomsky, 1977; Gorski and Rozman, 1987; Henry and Gasiewicz, 1987; Sewall *et al.*, 1994), suggesting that TSH is not responding to serum T_3. Continuous elevation of serum TSH levels following chronic TCDD treatment results in increased volume of thyroid follicular cells (Sewall *et al.*, 1994), followed by hyperplasia and increased thyroid weight (Andrae and Greim, 1992). Prolonged stimulation by TSH may ultimately lead to thyroid neoplasia (Hill *et al.*, 1989).

Thyroxine is metabolized (mostly in the liver) by isoform 1 of UDP-glucuronosyltransferase (UGT) to a glucuronide which is excreted in the bile (Bastomsky, 1977). Administra-

[1] Parameter values are from Kohn *et al.* (1993) unless otherwise specified. Where noted, five parameters from this earlier model were adjusted to improve the fit to experimental data.

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tion of TCDD to rats increases the hepatic activity of this enzyme (Bastomsky, 1977; Henry and Gasiewicz, 1987) by an aryl hydrocarbon (Ah) receptor-dependent mechanism (Bock, 1991). As secretion of TSH from the pituitary is increased by lowering serum T_4, induction of UGT might be responsible for the decrease in serum T_4 (Barter and Klaassen, 1992a) and the increase in serum TSH consequent to TCDD treatment. In order to determine if this mechanism is compatible with current knowledge about the regulation of these hormones, a physiologically based model of the above processes was constructed and its predictions compared with experimental data.

METHODS

The present model is an extension of the physiologically based model of Kohn *et al.* (1993) for the tissue distribution and metabolism of TCDD and its effects on several liver proteins. The strategy employed was to extend this model by including separate compartments for organs involved in the production, storage, and metabolism of thyroid hormones and equations for the rates of the biochemical processes involved. Interpretation of a large number of experimental observations was necessary during the course of the model construction process. What follows is a description of the model and the reasons for the choices made in its construction. A flowchart of the TCDD distribution portion of the extended model is given in Fig. 1, and a detailed flowchart of the thyroid hormone regulation portion is given in Fig. 2. Parameter values used in the model and their sources are listed in the Appendix. A listing of the program describing the model is available from the first author by electronic mail (kohn@phantom.niehs.nih.gov).

Modeling TCDD distribution. The model of Kohn *et al.* (1993) was extended by addition of compartments for the pituitary and thyroid. Because brown fat has a significant concentration of T_3 receptors, it was separated from the rest of the fat as another compartment. The GI tract and kidney were separated from the rapidly perfused compartment (termed "viscera") in the revised model in order to represent intestinal absorption and biliary and urinary excretion more realistically. Thyroid hormones are taken up into tissues by high-capacity carrier-mediated processes (Krenning *et al.*, 1981), which could result in depletion of extracellular hormones relative to the general circulation.

Andersen *et al.* (1993) developed a pharmacokinetic model of the disposition of TCDD in the rat in which compartments were divided into tissue and "tissue blood" spaces. This model could fit experimental data for liver and fat TCDD levels as a function of dose without requiring binding of TCDD to serum proteins as was done in their previous model (Leung *et al.*, 1990). This behavior is attributable to gradients in TCDD concentration between arterial and tissue blood spaces. Therefore, in this model each tissue compartment was associated with its own capillary space.

Instead of simply introducing tissue blood compartments and leaving the blood compartment unchanged as was done by Andersen *et al.* (1993), in this model the blood was distributed among the major vessels and the capillary beds of the individual tissue compartments. The measured tissue blood volumes of Altman and Dittmer (1971) were used in this model rather than the estimates of Bischoff and Brown (1966) as in the Andersen *et al.* model. Delivery of material from the blood in the major vessels to capillary beds was treated as flow limited. Rates of uptake from the capillaries into the tissues was restricted according to the permeability-surface area product of Andersen *et al.* (1993), referred to here as a "transport factor." This quantity was multiplied by the tissue blood flow rate, yielding expressions that are identical to the "diffusional clearances" of Kedderis *et al.* (1993).

The forms of the resulting equations for delivery of TCDD to capillary beds ($v_{delivery}$) and its net uptake (v_{uptake}) by tissues are

$$v_{delivery} = Q_{tissue} \times A_{blood}/V_{blood} - Q_{tissue} \times A_{tissue\ blood}/V_{tissue\ blood}$$

$$v_{uptake} = T_{tissue} \times Q_{tissue} \times A_{tissue\ blood}/V_{tissue\ blood} - T_{tissue} \times Q_{tissue} \times A_{tissue}/(P_{tissue} \times V_{tissue}),$$

where Q_i is the blood flow rate through tissue i, A_i is the amount of material in compartment i, V_i is the volume of compartment i, T_i is the transport factor between tissue i and its associated capillary blood, and P_i is the corresponding tissue:blood partition coefficient. Revised partition coefficients were taken from Andersen *et al.* (1993). The fat:blood partition coefficient and the transport factors were adjusted to fit the 91-day time courses of Abraham *et al.* (1988) for TCDD in livers and fat of rats given a single subcutaneous injection of TCDD in dimethyl sulfoxide/toluene.

Previous dosimetric models for TCDD treated its metabolism as proceeding with a pseudo-first-order rate constant that was scaled by (body weight)$^{-0.3}$. This representation was replaced in this model by a Hill equation for the kinetics of the metabolizing enzyme because the Hill equation is an empirical representation that can model a wide range of catalytic mechanisms. The substrate for the enzyme was treated as unbound liver cytosolic TCDD. The Hill parameters were optimized with the "praxis" algorithm (Brent, 1973) in the SCoPfit program (Simulation Resources, Inc., Berrien Springs, MI) to fit the temporal profiles of liver and fat TCDD in rats given a single oral dose (Abraham *et al.*, 1988). The Hill exponent was also adjusted to reproduce the dose–response data for rats given biweekly oral doses of TCDD (Tritscher *et al.*, 1992) in addition to matching the time courses of Abraham *et al.*

The BrdU labeling index observed in livers of rats treated with biweekly oral doses of TCDD corresponds to a tissue growth rate more than double that observed (Lucier *et al.*, 1991). Maronpot *et al.* (1993) found evidence of cytotoxicity (cellular swelling, vacuolization, and degeneration) and loss of plasma membrane integrity (leakage of liver enzymes into serum) in these rats. These measures exhibited the same dose–response curve shape as did the labeling index, and the histological signs of toxicity appeared only in the unlabeled cells. These observations suggest that the labeling index represents the net growth from hepatocellular proliferation minus death of cells injured by TCDD.

In order to reproduce tissue concentrations of TCDD following both short- and long-term repeated exposures, the model from which the current model is derived (Kohn *et al.*, 1993) required loss of TCDD by cell lysis (or at least leaky membranes) due to cytotoxic effects of cumulative exposure. However, the fate of this material was left unspecified. TCDD lost from lysed cells should appear in the interstitial fluid of the liver. Some of this TCDD may be taken up by nearby intact hepatocytes. The rest of this TCDD is expected to drain into the bile and be either reabsorbed into the blood or excreted in the feces. The revised model includes an equation for the net rate of secretion of TCDD into the bile by this mechanism. This material is treated as being transferred to the gut lumen, taken up into the GI tract blood compartment, and redistributed to the tissues.

The kinetics of hepatic expression of cytochromes P450 1A1 (CYP1A1) and 1A2 (CYP1A2) was represented by Michaelis–Menten equations in the previous model, treating the Ah–TCDD complex as the "substrate" of the rate-limiting step. Subsequently, data for production of *CYP1A1* mRNA and protein became available (Vanden Heuvel *et al.*, 1994), and these data were used to model the kinetics of *CYP1A1* mRNA production. The mechanism corresponding to the best-fitting model involved two DNA binding sites with different affinities for the liganded Ah receptor (Vanden Heuvel *et al.*, 1994; Kohn *et al.*, 1994b). Occupancy of both sites is required for transcriptional activation in this model, and formation of such a DNA–receptor complex is treated as rate limiting. To fit *CYP1A1* mRNA production at low doses, a small amount of this material was treated as protected from degradation by ribonuclease as a result of binding of the mRNA to ribosomes or other cellular material. CYP1A1 protein production was fit adequately by a Hill equation with *CYP1A1* mRNA treated as the "substrate" of the rate-limiting step (Kohn *et al.*, 1994b). This mathematical representation was used in the current model as well.

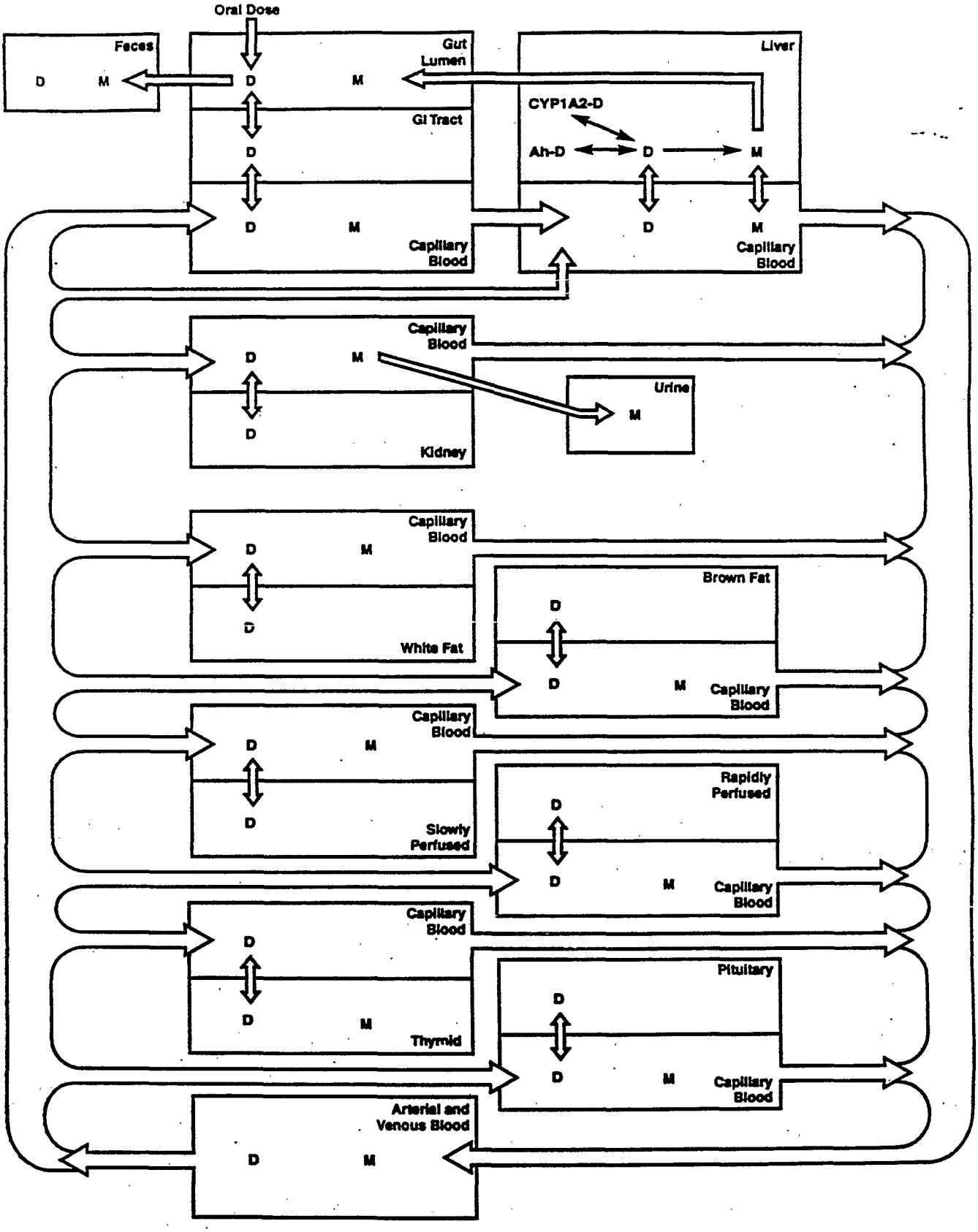

FIG. 1. Flowchart of the dosimetric model. Hollow arrows denote flows; solid arrows denote biochemical effects. Abbreviations: D, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; M, TCDD metabolite.

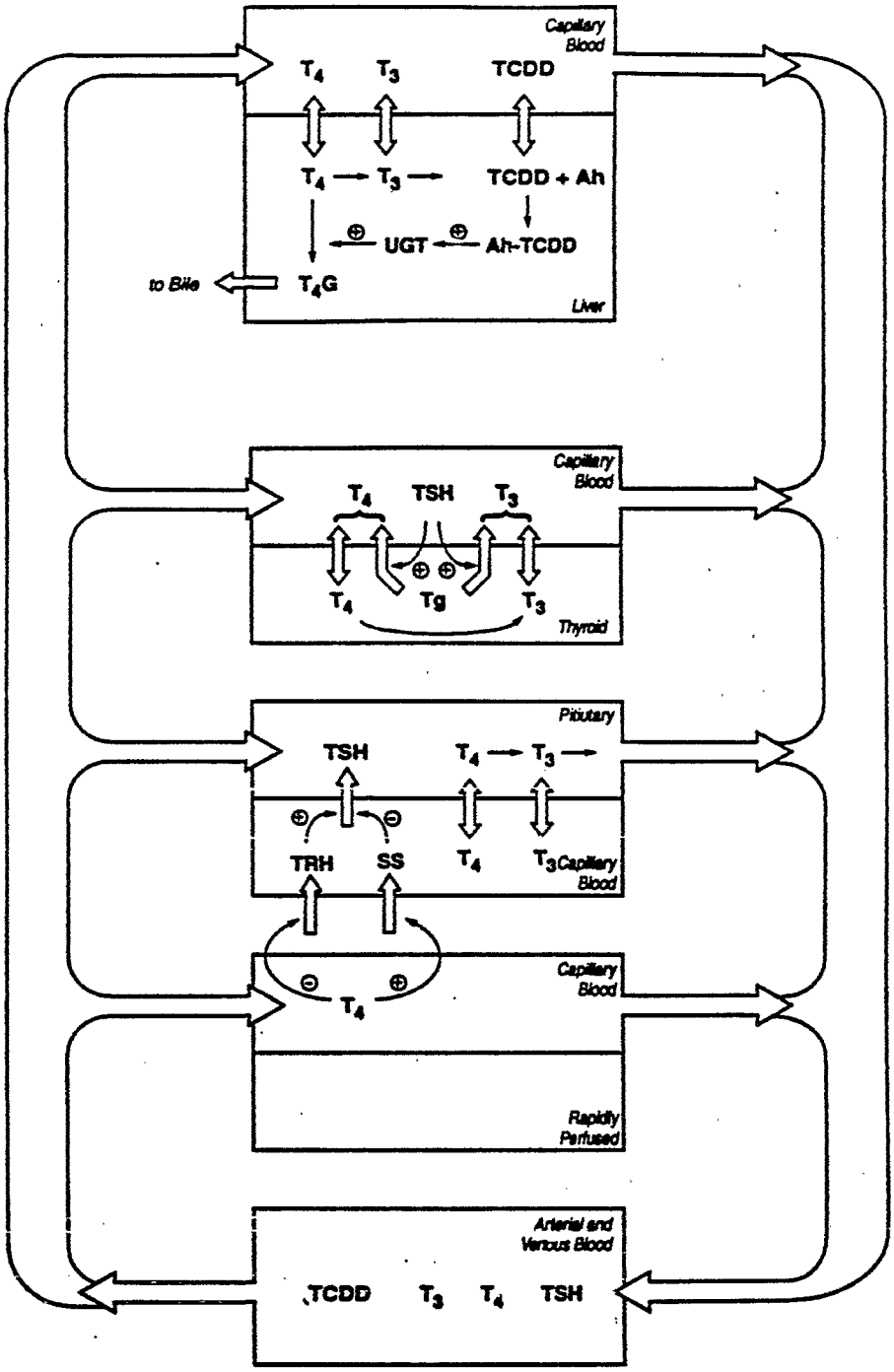

FIG. 2. Detail of the portion of the model dealing with thyroid hormone regulation. Hollow arrows denote flows; solid arrows denote biochemical effects. Abbreviations: Tg, thyroglobulin; T_4, thyroxine; T_3, 3,5,3′-triiodothyronine; TSH, thyrotropin; TRH, thyrotropin releasing hormone; SS, somatostatin; UGT, UDP-glucuronosyltransferase-1; T_4G, thyroxine glucuronide.

This representation is the simplest one that gives an adequate fit to the CYP1A1 induction data of Vanden Heuvel *et al.* (1994) and Tritscher *et al.* (1992). As Fisher *et al.* (1990) found at least four enhancer sequences associated with the *CYP1A1* gene, it is possible that more extensive experimental data would require a model with more complicated dependence on binding of Ah receptor complexes to enhancers. However, this portion of the model affects only the predicted amount of *CYP1A1* induction.

The model of Leung *et al.* (1990) treated the concentrations of TCDD bound to blood protein as a fixed multiple of the concentration of unbound TCDD. A revised version of this model (Andersen *et al.*, 1993) that treated

uptake of TCDD by tissues as "diffusion limited" and replaced the algebraic equation for induction of CYP1A1 by a differential equation reproduced observed liver and fat TCDD concentrations without considering binding to serum proteins. The model of Kohn *et al.* (1993) reproduced measured blood TCDD levels by including an algebraic equation for the blood concentration of TCDD-binding protein. That algebraic equation was replaced by a differential equation in the present model. An empirical equation (no mechanism is implied) for the rate of production of the protein was developed, and the protein was modeled as appearing in the liver capillary blood.

$$\nu_{\text{production}} = \frac{V_{\max}}{1 + [\text{TCDD}]_{\text{liver}}/K_i}.$$

$V_{\max}$ and K_i are not both identifiable from the dose–response of blood TCDD alone; a wide range of values yields the same $\nu_{\text{production}}$ rate. Therefore, the basal protein concentration (i.e., uninhibited production) estimated in the previous model (Kohn *et al.*, 1993) was used as an additional constraint on the maximal velocity. Free and bound TCDD and free protein were distributed to and equilibrated within all blood compartments, and free and TCDD-bound protein were subject to proteolytic degradation in all compartments.

Modeling regulation of thyroid hormones. T_3 and T_4 are formed as amino acid residues of thyroglobulin (Tg) in a ratio of 0.08–0.1 (Nunez and Pommier, 1982), and this protein is stored as a colloid in the thyroid follicular lumen. Secretion of the hormones occurs by pinocytosis of a portion of the colloid, hydrolysis of Tg inside the lysosomes, and exocytosis of the contents of the lytic vesicles (Harper *et al.*, 1977). Data for isolated perifused thyroid glands were used to calculate the basal (Tal *et al.*, 1986) and maximal (Tajima *et al.*, 1985) rates of T_4 secretion. Hotta *et al.* (1991) determined a ratio of 11.2 for the rates of secretion of T_4 and T_3, which is the ratio of the corresponding iodothyronines in Tg. The basal and TSH-stimulated rates of T_3 secretion were estimated by dividing the T_4 release rates by this ratio, yielding a maximal T_3 release rate comparable to that observed by Tajima *et al.* (1985). The basal rates were constants in the model; the TSH-stimulated rates were modeled by hyperbolic kinetics with respect to the concentration of TSH in the thyroid capillary blood. The effective binding constant of TSH was adjusted to permit the model to reproduce the levels of thyroid hormones in the blood of control rats.

Secretion of TSH from the pituitary gland is regulated by hypothalamic peptides (Spira and Gordon, 1986). These peptides, thyrotropin releasing hormone (TRH) and somatostatin (SS), are released from the hypothalamus into its local blood supply and delivered directly to the pituitary by a small vascular portal system (Labrie *et al.*, 1978). TSH secretion is stimulated by TRH, and this stimulated release is inhibited by SS. Assuming saturation kinetics for TSH release, the corresponding rate equation used in the model is

$$\nu_{\text{TSH release}} = \frac{V_{\max}^{\text{TSH release}}}{\left(\frac{K_a^{\text{TRH}}}{[\text{TRH}]} + 1\right) \times \left(\frac{[\text{SS}]}{K_i^{\text{SS}}} + 1\right)},$$

where [TRH] and [SS] indicate the peptides' respective concentrations in the pituitary blood. The maximal release rate and the binding constants of the peptides were obtained from the literature (see Appendix). TSH was modeled as appearing in the pituitary blood and being distributed with flow limited kinetics to all other blood compartments.

TSH release is known to be inhibited by thyroxine, although the direct effects of T_4 on the pituitary—e.g., synthesis of TRH receptors (Gershengorn, 1983)—require protein synthesis (Spira and Gordon, 1986). For simplicity, the shorter-term effects were assumed to arise from hyperbolic inhibition of TRH release and hyperbolic stimulation of SS release by T_4 in the brain blood (i.e., its concentration in the viscera blood compartment in this model). The reciprocal effects of serum T_4 on these hypothalamic peptides and the consequent effect on TSH release from the pituitary is a possible mechanism for control of the concentrations of circulating thyroid hormones.

$$\nu_{\text{TRH release}} = \frac{V_{\max}^{\text{TRH release}}}{[\text{total } T_4]/K_i^{T_4} + 1} \qquad \nu_{\text{SS release}} = \frac{V_{\max}^{\text{SS release}}}{K_a^{T_4}/[\text{total } T_4] + 1}.$$

The maximal rate of SS release was estimated from data for cultured hypothalamic cells (Shoemaker *et al.*, 1983); the maximal rate of TRH release was an adjustable parameter. In the absence of any kinetic data for SS binding, the T_4 activation constant for SS release was assumed to equal its inhibition constant for TRH release, and these quantities were set equal to the serum total T_4 concentration giving half-maximal suppression of TSH release (Gershengorn, 1983). The peptides were modeled as appearing in the pituitary blood compartment. Because the half-life of plasma TRH is very short (Spira and Gordon, 1986), hydrolysis of the hypothalamic peptides was modeled as occurring mainly in the pituitary blood. The observed half life for TRH was used for SS and TSH as well.

Thyroxine in the blood of rats is mostly bound to thyroxine-binding prealbumin and albumin; T_3 is mostly bound to albumin (Pardridge, 1981). Blood concentrations of these proteins and their binding constants for T_4 were obtained from Sutherland and Brandon (1976). The albumin binding constant for T_3 was taken as five times that of T_4 (Robbins and Johnson, 1979). Transport of thyroid hormones (bound and free), their binding proteins, and TSH between arterial plus venous blood and capillary blood was modeled as flow limited.

Administration of TCDD was found to lead to a dose- and time-dependent reduction in the amount of prealbumin in the blood (Albro *et al.*, 1978). The rate of production of this protein was modeled as inhibited by unbound liver TCDD with hyperbolic kinetics.

$$\nu_{\text{production}} = \frac{V_{\max}^{\text{production}}}{[\text{TCDD}]/K_i + 1},$$

where the value of $V_{\max}^{\text{production}}$ was set to reproduce the amount of prealbumin in control rats and K_i is an adjustable parameter. This equation is merely an empirical representation of the effect of TCDD on the production of prealbumin; no mechanism is implied.

Modeling uptake and metabolism of thyroid hormones. Uptake of thyroid hormones into rat hepatocytes is mediated by two active transport systems, one with high affinity but low capacity and the other with lower affinity but high capacity (Krenning *et al.*, 1981). Only unbound hormone in the tissue capillary blood was treated as available for uptake (Pardridge, 1981; Ekins, 1986; Mendel *et al.*, 1989). Because the higher-affinity system apparently carries both T_3 and T_4 (Krenning *et al.*, 1981), transport of each hormone was treated as competitively inhibited by the other hormone. For example, uptake of T_4 was represented by the simplified rate equation

$$\nu_{T_4\text{ uptake}} = \frac{V_{\max}^{T_4}\left(1 - \frac{[T_4]_{in}/[T_4]_{out}}{P}\right)}{\frac{K_m^{T_4}}{[T_4]_{out}}\left(1 + \frac{[T_4]_{in}}{K_m^{T_4}} + \frac{[T_3]_{out}}{K_m^{T_3}} + \frac{[T_3]_{in}}{K_m^{T_3}}\right) + 1},$$

where $V_{\max}^{T_4}$ is the maximal T_4 uptake rate, P is corresponding tissue:blood partition coefficient, and in and out refer to the cytosolic and capillary blood concentrations, respectively. The equation for T_3 transport has the roles of T_3 and T_4 interchanged. T_3 and T_4 have separate low-affinity carriers which do not show inhibition of each other's transport (Krenning *et al.*, 1981). The corresponding rate equation is similar to that of the high-affinity transporter, except that the denominator terms for inhibition by the other hormone are absent.

Transfer of hormone from the vascular space to tissues is restricted by the limited permeability of the capillary endothelium to plasma proteins. Thus, muscle, with a continuous endothelium, equilibrates with plasma in about 3 hr, whereas liver or kidney, with discontinuous or fenestrated endothelia, equilibrate in 15 min (Oppenheimer and Surks, 1974). The kidney was used as a model for pituitary and thyroid as all these organs have fenestrated capillary endothelia and would be expected to show similar permeability to the protein-bound hormones. The lower uptake rate for the brain was used as the model for other tissue compartments (e.g., muscle) as they have continuous capillary endothelia and would be expected to have similar permeabilities. Krenning *et al.* (1981) reported maximal uptake rates of thyroid hormones per microgram of DNA. Estimates of the maximal uptake rates in other tissues were calculated from reported mg DNA/g tissue values (Oppenheimer, 1983). The K_ms determined for the liver were used for all tissues.

Octanol:water partition coefficients (K_{ow}) values for T_3 and T_4 (Pardridge and Mietus, 1980) were converted to $P_{oil:water}$ partition coefficients, using the regression equation of Lyman *et al.* (1990). From the linear free energy relationship for the solubility of aromatic compounds in various solvents given by Abraham *et al.* (1985), the relationship

$$\log_{10} P_{oil:blood} = 0.588 \times \log_{10} P_{oil:water} + 0.295$$

was derived. $P_{fat:blood}$ values were assumed to equal $P_{oil:blood}$ values, and tissue:blood partition coefficients for the other tissue groups in the model were estimated from $P_{fat:blood}$ by use of the regression equations of Fiserova-Bergerova and Diaz (1986).

Thyroid-sensitive tissues contain T_3- and T_4-binding proteins. This model includes binding of thyroid hormones to these proteins in pituitary, brown fat, liver, kidney, and the rapidly perfused tissues compartment. These tissues constitute 10.3% of the body weight in this model, and the blood constitutes an additional 5.4% of the body weight. The volume of distribution of thyroxine in the adult rat is 15.6% of the body weight (Van Middlesworth, 1974), suggesting that tissues that are less sensitive to thyroid hormones accumulate little hormone. Some intracellular T_3 is bound to a receptor localized in the nucleus of these tissues (Latham *et al.*, 1978). Tissue contents of the T_3 receptor were taken from Oppenheimer (1983).

The binding constant for the purified receptor (Aprilletti *et al.*, 1987) was used in this model. Ramsden (1978) described a 70-kD thyroxine-binding cytosolic protein in liver and kidney. Maximal binding capacities for this protein were obtained from Barnes and DeGroot (1983) for liver and from Segal and Ingbar (1986) for other tissues (see Appendix). There are two types of T_4-binding site on this protein, but the higher-affinity site has been reported as having "limited capacity" (Ramsden, 1978). Therefore, the binding constant for the lower-affinity site (Ramsden, 1978) was used in this model for all thyroid-sensitive tissues.

Thyroid hormones taken up by the above tissues are subject to deiodination by a suite of at least four distinct enzymes. Type I T_4 5′-deiodinase is a selenocysteine enzyme found in liver and kidney (Chopra, 1991; Berry and Larsen, 1992) which catalyzes the glutathione-dependent deiodination of T_4 (Goswami and Rosenberg, 1985). This enzyme is also found in the thyroid (Leonard and Visser, 1986). In addition, the liver contains a low K_m isoform of Type I deiodinase (Chopra, 1991). Other tissues contain a distinct Type II T_4 5′-deiodinase (Leonard and Visser, 1986) with a much greater affinity for T_4 than the Type I enzyme (Berry and Larsen, 1992). These activities were included in the brown fat, pituitary, and the viscera compartment. Michaelis–Menten kinetics were assumed for all three of these enzymes. Values of the kinetic constants were taken from the literature.

Type III deiodinase is specific for removal of iodine from the inner ring of iodothyronines (Leonard and Visser, 1986) and is responsible for conversion of T_4 into 3,3′,5′-triiodothyronine ("reverse" T_3, rT_3) and of T_3 into 3,3′-diiodothyronine. Because so little T_4 is converted to rT_3—its serum concentration is typically about 10% of that of T_3 (Gorski and Rozman, 1987)—this enzyme was modeled as a T_3-deiodinase which is competitively inhibited by T_4 and production of rT_3 was ignored. The apparent K_i for T_4 (Leonard and Visser, 1986) was used as an estimate of its inhibition constant. Eltom *et al.* (1992) found that administration of TCDD to rats decreases 5′-deiodinase activity although this effect is not reflected in decreased production of T_3. Their suggestion that TCDD inhibits inner-ring deiodination was included in this model as competitive inhibition of binding of T_3. The corresponding rate equation used in the model is

$$v_{deiodination} = \frac{V_{max}^{deiodination}}{\frac{K_m^{T_3}}{[T_3]}\left(\frac{[T_4]}{K_i^{T_4}} + \frac{[TCDD]}{K_i^{TCDD}} + 1\right) + 1},$$

where $[T_3]$, $[T_4]$, and $[TCDD]$ refer to the concentrations of chemical not bound to protein. This rate equation was included in the liver, kidney, and viscera compartments. Values of the K_m for T_3 from 2.3 nM to 6.2 μM have been reported (Leonard and Visser, 1986). Because of the large uncertainty in the value of the K_m, this kinetic constant was treated as a parameter in the model and its value adjusted to reproduce the total T_3 concentration observed in the blood of control rats.

The deiodinases are microsomal enzymes whose activities were measured per milligram of microsomal protein recovered from various tissues. Fouts and Devereux (1973) have shown that only half of the activity of microsomal enzymes in whole tissue homogenates is typically recovered in isolated microsomes. Their results indicate that there is 35 mg of microsomal protein/g liver and 16 mg/g lung. Their lung value was used for the rapidly perfused tissues compartment in this model. Coughtrie *et al.* (1987) isolated 9 mg of microsomal protein/g kidney. A 50% yield was assumed for this tissue as well. These values were used to calculate V_{max} values from the reported specific activities of the deiodinases in microsomal preparations.

Transcription of the *UGT1* gene into mRNA was modeled as proceeding with Michaelis–Menten kinetics, treating binding of the liganded Ah receptor as the rate-limiting step. The constitutive rate of UGT expression, the V_{max} of transcription, and the apparent K_m of the Ah–TCDD complex were optimized with the SCoPfit program to reproduce the *UGT* mRNA concentration 4 days following a single oral dose of TCDD (Vanden Heuvel *et al.*, 1994). This experiment was selected for the reference data because the doses used spanned more than five orders of magnitude. As was found in modeling of the transcription of the *CYP1A1* gene (Vanden Heuvel *et al.*, 1994), a small amount of the *UGT* mRNA had to be treated as protected from degradation by ribonuclease, perhaps due to binding to ribosomes or other structural elements of the cell. The equation for mRNA degradation used in the model is

$$v_{degradation} = k_{degradation}([UGT\ \text{mRNA}] - \text{protected mRNA}),$$

where $k_{degradation}$ is the mRNA degradation rate constant established for *CYP1A1* (Vanden Heuvel *et al.*, 1994).

Synthesis of UGT protein on the mRNA template was modeled as proceeding with Michaelis–Menten kinetics, treating binding of the *UGT* mRNA to ribosomes as the rate-limiting step. The V_{max} of transcription and the apparent K_m of the mRNA were adjustable parameters. The UGT turnover number was calculated from the specific T_4-UDP-glucuronosyltransferase activity of the purified protein (Coughtrie *et al.*, 1987). The V_{max} of T_4 glucuronidation is the product of protein concentration and turnover number and varies with dose of TCDD and time. As the reaction kinetics of T_4 glucuronidation have not been studied, the apparent K_m of T_4 was an adjustable parameter in the model. The hepatic UGT concentration in control rats was calculated from the data of Barter and Klaassen (1992b).

Saito *et al.* (1991) found that microsomes from rats treated with β-naphthoflavone, 3-methylcholanthrene, or polychlorinated biphenyls exhibited comparable increases in T_3 and T_4 glucuronidation relative to those activities in untreated rats. However, Bastomsky (1977) observed TCDD to increase the biliary excretion of T_4 but not T_3. Even if the UGT substrate

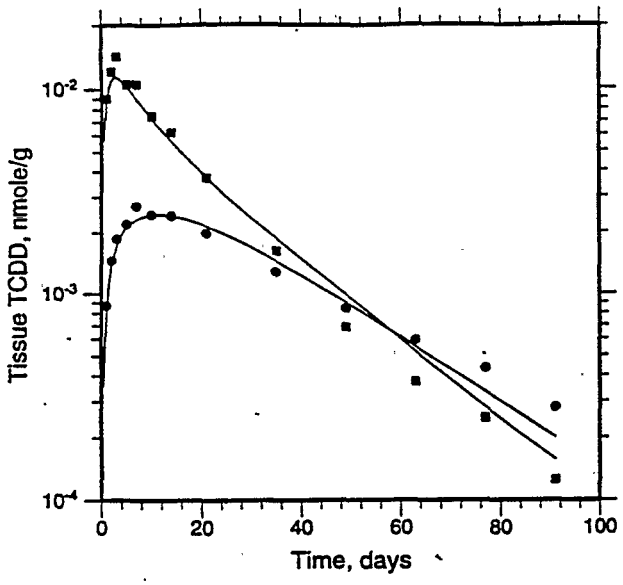

FIG. 3. Fit of the physiological dosimetric model to the time course data of Abraham *et al.* (1988). Filled squares are the data points for liver TCDD, filled circles are for adipose tissue.

binding site has comparable affinity for T_4 and T_3, because the hepatic T_4 concentration is very much greater than that of T_3, most of the UGT binding sites will be occupied by T_4, resulting in insignificant T_3 glucuronidation. Therefore, the model does not include T_3 glucuronidation.

The fully assembled model, comprising 186 differential equations, was implemented in the SCoP simulation language (Kootsey *et al.*, 1986; Kohn *et al.*, 1994a) and solved with a C language translation of the LSODA implementation of the Gear algorithm (Lawrence Livermore Laboratory). The model was used to simulate the experiments in which female Sprague–Dawley rats were given biweekly oral doses of 0.35–125 ng TCDD/kg body weight/day in corn oil for 31 weeks (Tritscher *et al.*, 1992). After this time, the rats were killed and the blood levels of T_4, T_3, and TSH and hepatic *UGT* mRNA were measured (Sewall *et al.*, 1994).

RESULTS

A comprehensive dosimetric model of the distribution of TCDD among tissues in the rat, its metabolic clearance, and its effect on several hepatic proteins was extended to include the regulation of serum thyroid hormones and TSH by the hypothalamic–pituitary–thyroid axis. Feedback inhibition of hormonal release from the thyroid was modeled by a simplified empirical scheme. Serum T_4 was assumed to inhibit secretion of TRH while activating secretion of SS from the hypothalamus by an unspecified mechanism. Release of TSH from the pituitary was modeled as regulated by reciprocal effects of these hypothalamic peptides, and thyroid hormone secretion was modeled by hyperbolic dependence on the blood TSH concentration. Induction of hepatic UGT mediated by the Ah–TCDD complex increases clearance of T_4 in the model, leading to altered calculated concentrations of the hypothalamic peptides and increased rates of TSH secretion. Parameter values which produced a fit to the data of Tritscher *et al.* (1992) and Sewall *et al.* (1994) are given in the Appendix.

The first step in verifying the reliability of the model was checking that it reproduces the observed TCDD distribution. This fit depends on the parameters for TCDD uptake into tissues and its metabolism in the liver. Next it was verified that the model reproduced the dose–response of hepatic proteins (which depends on the values of gene expression parameters in the model) at least as well as did the original model. Then the model's predictions of blood hormone levels (depending on hormone release and metabolism parameters) and hepatic UGT activity (depending on UGT expression parameters) were compared to their observed values.

TCDD Distribution and Metabolism

Andersen *et al.* (1993) increased the fat:blood partition coefficient from its original value of 350 (Leung *et al.*, 1990) to 375 in their revised model. A value of 425 in the present model gave an optimal fit to the temporal tissue TCDD data of Abraham *et al.* (Fig. 3). The resulting fit to their measured adipose tissue TCDD concentrations was substantially improved over the model of Kohn *et al.* (1993). The fit to their measured liver concentrations was improved at short times and was only insignificantly worse at long times compared to that of the previous model. The present model also reproduces (Table 1) the observed dose–responses of liver and fat TCDD concentrations 7 days following single subcutaneous injections of TCDD in dimethyl sulfoxide/toluene (Abraham *et al.*, 1988). Hill parameters for TCDD metabolism showed slightly sigmoidal kinetics (the optimal Hill exponent was

TABLE 1
Tissue TCDD Concentrations 7 Days Following Single Subcutaneous Injections[a]

Dose, ng/kg	Liver	Fat
1	1.56×10^{-5}	9.78×10^{-6}
	$(9.63 \times 10^{-6} \pm 2.80 \times 10^{-6})$	(0)
3	4.33×10^{-5}	2.94×10^{-5}
	$(3.17 \times 10^{-5} \pm 6.21 \times 10^{-6})$	$(4.32 \times 10^{-5} \pm 4.66 \times 10^{-6})$
10	1.39×10^{-4}	9.62×10^{-5}
	$(1.26 \times 10^{-4} \pm 3.76 \times 10^{-5})$	$(1.53 \times 10^{-4} \pm 2.61 \times 10^{-5})$
30	4.49×10^{-4}	2.78×10^{-4}
	$(5.03 \times 10^{-4} \pm 9.94 \times 10^{-5})$	$(4.32 \times 10^{-4} \pm 6.52 \times 10^{-5})$
100	0.00201	8.58×10^{-4}
	$(0.00217 \pm 4.04 \times 10^{-4})$	$(0.00104 \pm 2.02 \times 10^{-4})$
300	0.00897	0.00232
	$(0.0105 \pm 6.83 \times 10^{-4})$	$(0.00254 \pm 2.33 \times 10^{-4})$
1000	0.0405	0.00727
	(0.0332 ± 0.00683)	$(0.00627 \pm 5.28 \times 10^{-4})$
3000	0.133	0.0229
	(0.0866 ± 0.00745)	$(0.0114 \pm 9.63 \times 10^{-4})$

[a] Concentrations in nmol/g. Observed mean values ± standard deviations from Abraham *et al.* (1988) in parentheses.

TABLE 2
Tissue TCDD Concentrations Following Repeated Oral Doses (5 Days/Week)[a]

	Dose, ng/kg		
	10	100	1000
1 week			
Liver	0.000972 (0.0)	0.0146 (0.00870–0.0155)	0.165 (0.143–0.165)
Fat	0.000365 (0.0)	0.00260 (0.000932–0.00466)	0.0239 (0.0217–0.0404)
3 weeks			
Liver	0.00230 (0.00217–0.00280)	0.0385 (0.0298–0.0435)	0.345 (0.227–0.457)
Fat	0.00103 (0.000932)	0.00729 (0.00590–0.0109)	0.0910 (0.0497–0.0963)
5 weeks			
Liver	0.00367 (0.00342–0.00652)	0.0553 (0.0519–0.0711)	0.431 (0.471–0.796)
Fat	0.00168 (0.000620–0.00124)	0.0146 (0.0118–0.0161)	0.196 (0.0767–0.305)

[a] Concentrations in nmol/g tissue. Range of observed values (Rose *et al.*, 1976) in parentheses.

1.12). Fixing the Hill exponent at 1.0 (i.e., Michelis–Menten kinetics) had little effect on the fit to the data of Abraham *et al.* (1988) but resulted in a significantly worse fit to the dose–response data for TCDD concentration of Tritscher *et al.* (1992).

At a dose of 125 ng/kg/day, the calculated unbound TCDD concentrations in arterial blood, liver capillary blood, and hepatic tissue after 31 weeks of treatment were 0.792, 0.778, and 0.550 nM, respectively. The small computed gradient between arterial and tissue blood arises from the assumed flow limitation of delivery of TCDD from the general circulation and restricted uptake into the tissues (see below). The 7.05-fold gradient between capillary blood and tissue is considerably smaller than the liver:blood partition coefficient of 20, i.e., the distributions between blood and tissues are far from equilibrium. Estimates for the tissue transport factors which permitted reproduction of the liver and fat TCDD temporal profiles of Abraham *et al.* (1988) were all less than 1. This restriction on the rate of TCDD uptake into tissues is responsible for the relatively small calculated liver:blood gradient. These results suggest that uptake of TCDD into tissues is limited by transport across the cell membrane rather than by its rate of delivery to the tissue in agreement with Andersen *et al.* (1993).

The model computes significant biliary clearance of TCDD only after several months of chronic dosing with greater than 35 ng TCDD/kg/day, consistent with the observation of cytotoxicity only at these doses after 31 weeks of treatment (Maronpot *et al.*, 1993). This behavior permitted fitting tissue TCDD levels consequent to both short (Rose *et al.*, 1976)- and long-term (Tritscher *et al.*, 1992) repeated exposures. An alternative model which neglected this possible mechanism could not reproduce the data from all these experiments. As clearance of TCDD consequent to cytotoxicity is calculated to occur only after long-term exposure, detection of TCDD metabolites but not parent compound in the bile would be expected following short-term treatment.

The augmented dosimetric model predicted liver and fat TCDD concentrations of 0.0130 and 0.00525 nmol/g, respectively, 22 days following oral administration of a dose of 1 μg/kg in corn oil. The ranges of observed values (Rose *et al.*, 1976) were 0.00869–0.0145 and 0.00100–0.0222 nmol/g for liver and fat, respectively. The fit to these experimental data was significantly improved over the original formulation of this model. Similarly, the revised model was used to simulate the experiments of Rose *et al.* (1976) in which rats were given oral doses of TCDD in corn oil 5 days/week for 7 weeks. The resulting fit of calculated liver and fat TCDD concentrations to the experimental data (Table 2) was significantly improved over that obtained with the original model.

Tritscher *et al.* (1992) administered diethylnitrosamine (175 mg/kg ip) to female Sprague–Dawley rats and followed this 14 days later with biweekly oral doses of TCDD in corn oil for 31 weeks. The revised model's calculated blood TCDD concentrations for these experiments (Table 3) were comparable to those calculated by the original model and fit the data (A. M. Tritscher, NIEHS, unpublished results) well. An alternative model that ignored protein binding of TCDD provided a poor fit at low dose but a good fit at high dose (Fig. 4).

The calculated liver TCDD concentrations for the experiments of Tritscher *et al.* (Table 3) were also similar to those

TABLE 3
Tissue TCDD Concentrations Following Biweekly Oral Doses for 31 Weeks[a]

Dose, ng/kg/day	Blood, nM	Liver, nmol/g
0.1	0.00655	4.98×10^{-5} (5.47×10^{-5}–1.33×10^{-4})[b]
0.3	0.00798	0.000132 (9.19×10^{-4}–0.000206)[b]
1.0	0.00969	0.000393 (0.000404–0.000755)[b]
3.5	0.0133 (0.0127–0.0174)[b]	0.00134 (0.000652–0.00267)[c]
10.7	0.0211 (0.0143–0.0251)[b]	0.00460 (0.00435–0.00528)[c]
35.7	0.0419 (0.0323–0.103)[b]	0.0188 (0.0155–0.0311)[c]
125	0.103 (0.0401–0.134)[b]	0.0727 (0.0385–0.134)[c]

[a] Range of observed values in parentheses.
[b] A. M. Tritscher, NIEHS, unpublished results.
[c] Tritscher *et al.* (1992).

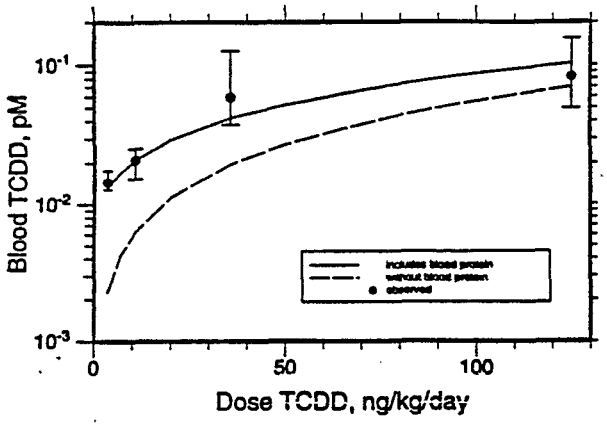

FIG. 4. Comparison of the dosimetric model's predictions for total blood TCDD with and without inclusion of serum TCDD-binding protein. Experimental data points (mean values) are unpublished results of A. M. Tritscher, NIEHS; error bars indicate the range of measured concentrations.

computed with the original model. The revised model's predictions for liver TCDD concentrations at very low doses are in fairly good agreement (Table 3) with measurements obtained since the construction of the original model (A. M. Tritscher, NIEHS, unpublished results). These new data were not used to estimate parameter values in the current study.

Concentrations of Hepatic Proteins

The responses of liver CYP1A1 and CYP1A2 proteins, commonly used as biomarkers for TCDD exposure, were computed with the model for biweekly oral dosing with TCDD (Tritscher *et al.*, 1992). The results (Table 4) were similar to those of the previous version of this model and gave a good fit to observed responses. The fit of the model to the hepatic Ah, estrogen, and plasma membrane EGF receptors is given in Table 5. These values are also similar to those from the previous version of the model. The submodels for production of CYP1A1 and for the decrease in plasma membrane EGF receptors have no effect on the computed distribution of TCDD. The submodels for CYP1A2 and the Ah and estrogen receptors affect the extent of protein binding of TCDD in the liver and, consequently, do affect the TCDD distribution.

TABLE 4
Computed Responses of Liver Cytochrome P450 Isozymes to Biweekly Oral Doses of TCDD for 31 Weeks[a]

Dose, ng/kg/day	CYP1A1	CYP1A2
0	0.0216 (0.00810–0.0351)	0.557 (0.352–0.714)
3.5	0.773 (0.269–0.953)	1.014 (0.840–2.31)
10.7	1.868 (1.89–3.13)	1.722 (1.39–3.56)
35.7	3.745 (2.91–4.47)	3.297 (2.39–4.21)
125	5.131 (3.72–5.99)	5.172 (2.86–10.3)

[a] Concentrations in nmol/g liver. Ranges of observed values (Tritscher *et al.*, 1992) in parentheses.

TABLE 5
Computed Responses of Hepatic Receptor Proteins to Biweekly Oral Doses of TCDD for 31 Weeks[a]

Dose, ng/kg/day	Ah receptor	Estrogen receptor	EGF receptor[b]
0	2.11 (2.1)[c] (2.1–3.2)[d]	5.16 (5.1)[c]	2.55 (2.06–2.61)
3.5	2.91	4.79	2.38 (1.42–2.95)
10.7	4.01 (3.0–5.7)[d]	4.30	2.12 (1.18–2.45)
30	5.83 (5.5–7.8)[d]	3.52	1.66
35.7	6.21	3.36	1.56 (0.889–1.64)
100	8.14 (4.9–10.5)[d]	2.61	0.984
125	8.52 (8.5)[f]	2.47 (2.3)[e]	0.873 (0.702–1.13)

[a] Concentrations in pmol/g liver. Ranges of observed values in parentheses.
[b] Sewall *et al.* (1993).
[c] Poland and Knutson (1982).
[d] Sloop and Lucier (1987).
[e] Clark *et al.* (1991).
[f] Maximal induction observed (Poland and Knutson, 1982).

Regulation of Thyroid Hormones

The computed time courses of blood T_4, T_3, and TSH for a dose of 125 ng/kg/day in the biweekly dosing experiments are given in Fig. 5. The curve shapes for the responses of the hormone levels are similar to those computed for liver and fat TCDD concentrations (Kohn *et al.*, 1993) in those experiments. Several computer experiments were conducted to identify the factors which are most important in regulating the blood concentration of these hormones.

For control rats, the model predicts that 98.2% of blood T_3 is bound to albumin, and 86.3% and 13.6% of blood T_4 are bound to prealbumin and albumin, respectively. The fractions of the hormones bound in blood are comparable to the values reported by Pardridge (1981) and Gorski and Rozman (1987). The model predicts that 96.1% of liver T_3 is bound to its nuclear receptor, and 95.7% of liver T_4 is protein bound. Similar results were obtained for the kidney.

The calculated concentrations of unbound T_3 in arterial blood and liver blood were 13.4 and 11.6 pM, respectively; the corresponding values for T_4 were 14.7 and 14.3 pM, respectively. The arterial:capillary gradients for these hormones are small; the calculated concentration ratios are 1.16 and 1.02 for T_3 and T_4, respectively. The maximal hepatic deiodination rates for T_3 and T_4 are more than an order of magnitude lower than the maximal hepatic uptake rates (see Appendix). Furthermore, the excess intracellular binding sites for thyroid hormones keeps their concentrations well below their K_ms for metabolism. The restricted rate of removal of the hormones leads to near equilibrium among the blood pools.

The model's computed dose-responses of T_4, T_3, and TSH are given in Table 6 and reproduce the data of Sewall *et al.* (1994) very well. The computed decrease in blood T_4 was dependent on induction of UGT. When TCDD inhibition of production of prealbumin (Albro *et al.*, 1978) was neglected, the model did not predict as great a decrease in the blood T_4 concentration as was observed (Sewall *et al.*, 1994). The computed increase in T_3 at higher doses of TCDD was likewise dependent on the specification of the model. An alternative model which neglected TCDD inhibition of Type III deiodinase resulted in only a slight difference in blood T_3 at the highest dose of TCDD compared to control rats. When competition by T_4 for that enzyme's binding site was neglected as well, the model predicted less T_3 in blood at the highest dose of TCDD than in controls. Another alternative model in which T_4 inhibited release of TSH by acting directly on the pituitary rather than on the hypothalamus predicted a twofold increase in circulating TSH instead of the observed 27% increase.

TABLE 6
Blood Hormone Concentrations after 31 Weeks of Biweekly Oral Dosing with TCDD[a]

Dose, ng/kg/day	T_4, nM	T_3, nM	TSH, pM
0.0	29.0 (26–32, 29)	0.760 (0.61–0.85, 0.75)	77.8 (36.1–162, 77.3)
0.1	28.9	0.760	78.0
0.35	28.7	0.759	78.2 (23.2–93.2, 50.6)
1.0	28.2	0.756	78.6 (22.5–77.5, 53.6)
3.5	27.0 (23–35, 27)	0.749	79.9 (32.1–117, 87.1)
10.7	24.7 (18–32, 24.7)	0.737	82.5
35.7	20.7 (12–21, 17.7)	0.737	87.8
125.0	15.2 (12.5–20, 16.5)	0.843 (0.55–1.10, 0.84)	97.9 (31.8–189, 98.5)

[a] Observed values (range, mean) from Sewall *et al.* (1994) in parentheses.

Observations of the kinetics of thyroid hormone metabolism and whole-body clearance have led to the concept that thyroid secretion contributes a minor portion of the circulating T_3; the majority comes from deiodination of T_4 (Oppenheimer and Surks, 1974). The high K_m Type I 5′-deiodinase is thought to provide circulating T_3 to other tissues, while the T_3 produced by the low K_m Type II 5′-deiodinase is considered to be destined for use within the tissue in which it is formed (Berry and Larsen, 1992). However, the extent of this conversion and the tissues which make the major contributions to circulating T_3 are not clear. Oppenheimer (1983) suggested that 15% of circulating T_3 comes from the thyroid and the rest from peripheral tissues—50% of the conversion occurring within slowly exchanging pools (e.g., brain and muscle) and 40% in the liver. Chanoine *et al.* (1993) have demonstrated that 55–60% of the circulating T_3 in rats originates in the thyroid and suggested that it most likely derives from deiodination of T_4.

The model predicts that 12.6% of the secreted T_4 is converted to T_3 in control rats, comparable to the 17% indicated by Oppenheimer and Surks (1974). Most of this conversion is computed to occur in the thyroid gland, which contributes 58.5% of the calculated total circulating T_3 in agreement with the results of Chanoine *et al.* (1993). T_3 produced by type I 5′-deiodinase of the liver and kidney, on the other hand, is not computed to be exported. Type II 5′-deiodinase

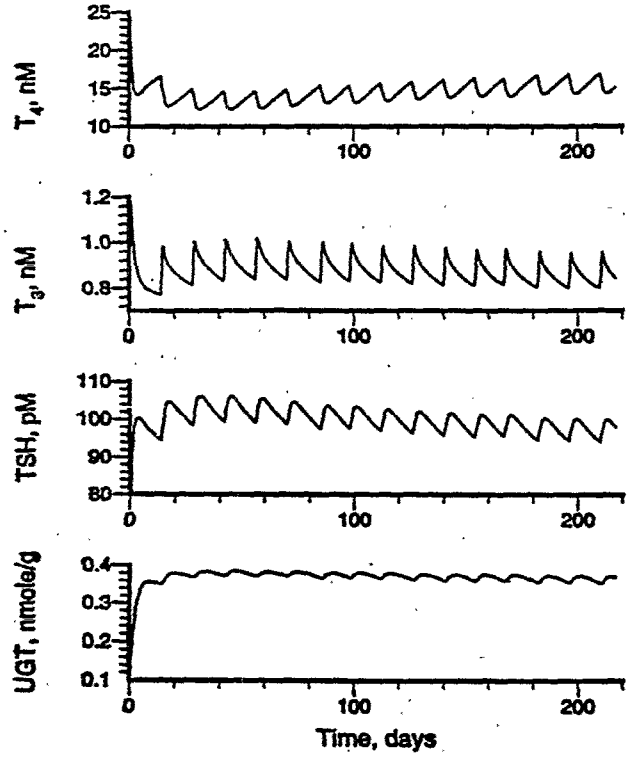

FIG. 5. Time course of blood T_4, T_3, TSH, and hepatic UGT protein computed by the model for biweekly oral dosing with TCDD equivalent to a dose rate of 125 ng/kg/day.

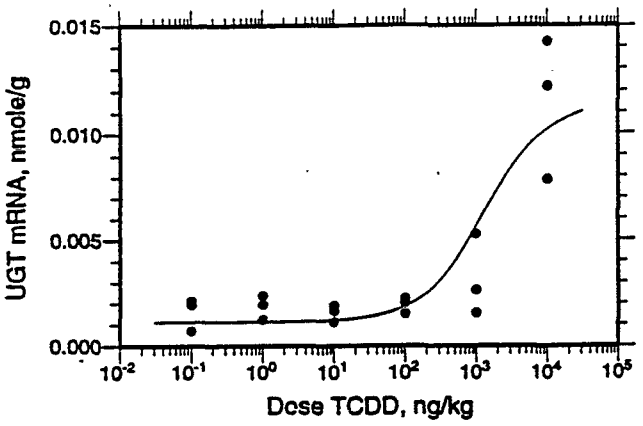

FIG. 6. Fit of *UGT* mRNA concentrations calculated by the model to the values observed (Vanden Heuvel *et al.*, 1994) 4 days following oral doses of TCDD.

of the pituitary is computed to produce more T_3 than is taken up from blood by this gland. These computations are consistent with the idea that nearly all of the hepatic and renal T_3 is taken up from blood whereas virtually all of the pituitary T_3 is produced locally (Larsen *et al.*, 1981).

Computed UGT Activity

The computed time course of liver UGT protein for a dose of 125 ng/kg/day is given in Fig. 5. This behavior is the result of induced transcription of *UGT* mRNA and its translation into protein. Formal optimization of the Michaelis–Menten parameters for this mRNA production utilized the data of Vanden Heuvel *et al.* (1994) from rat livers 4 days following oral dosing with TCDD. The fit to the *UGT* mRNA data of Vanden Heuvel *et al.* is shown in Fig. 6.

The experiments of Tritscher *et al.* (1992), 31 weeks of biweekly gavage with TCDD in corn oil, also were simulated with the model. The mRNA data from these experiments were not used in estimating the parameters in this model. The predicted responses of *UGT* mRNA and UGT protein are given in Table 7. The calculated amounts of *UGT* mRNA reproduce the observed values at low doses. The model underpredicts the mRNA levels at intermediate doses and overpredicts mRNA at the highest dose. Owing to large interindividual variability, the concentration ranges at the highest dose overlap those for control rats and it is difficult to ascertain the true shape of the dose–response curve. However, there is a tendency toward increasing message level with increasing dose similar to that computed by the model.

The model was used to simulate several other experiments, and the predicted induction of UGT activity was compared with that observed (Table 8). The model reproduces the observed induction at most TCDD doses in the range that was used to construct this model (<2 μg/kg). It underpredicted the induction at a dose of 5 μg/kg but reproduced the induction at a dose of 10 μg/kg. Owing to the lack of data, many processes in this model (e.g., production of binding proteins) were represented by greatly simplified empirical equations whose constants were adjusted to yield a good fit to the hormone dose–responses. These parameter values may not be sufficiently accurate for prediction of induction at doses higher than those used to estimate these quantities, and this limitation may be one reason for the poorer fits at high doses. However, this limitation would not affect the model's ability to extrapolate responses to lower doses.

DISCUSSION

The augmented dosimetric model described here was used to investigate the hypothesis that chronic exposure to TCDD

TABLE 7
UGT mRNA and Protein after 31 Weeks of Biweekly Oral Dosing with TCDD[a]

Dose, ng/kg/day	UGT mRNA, pmol/g	UGT protein, nmol/g
0.0	1.13 (0.747–4.03, 2.10)	0.118
0.1	1.15 (0.956–4.16, 2.78)	0.120
0.35	1.19 (0.234–2.19, 1.42)	0.123
1.0	1.27 (3.13–7.52, 4.82)	0.130
3.5	1.55 (3.75–5.57, 4.63)	0.153
10.7	2.31	0.201
35.7	4.62	0.291
125.0	8.79 (2.55–6.09, 3.88)	0.369

[a] Observed values (range, mean) from Sewall *et al.* (1994) in parentheses.

TABLE 8
Computed Induction of UGT Following Oral Doses of TCDD

Dose, μg/kg	Latency[a]	UGT, nmol/g	Calculated fold induction	Observed fold induction
0.0		0.116		
0.05	6	0.145	1.25	1.02[b]
0.2	3	0.199	1.72	1.38–2.57[c]
0.25	6	0.228	1.97	1.96[d]
1.0	1	0.167	1.44	1.53[d]
1.0	3	0.274	2.37	1.67–5.06[c]
1.0	6	0.326	2.82	3.10[d]
5.0	3	0.308	2.66	3.85–4.87[c]
5.0	6	0.378	3.27	5.88[d]
10.0	10	0.402	3.47	3.71[e]

[a] Days between dosing and sacrifice.
[b] Lucier *et al.* (1986).
[c] Lucier *et al.* (1973).
[d] Eltom *et al.* (1992).
[e] Bank *et al.* (1989).

results in induction of UGT by TCDD, depletion of serum T_4 by the activity of this enzyme, and sustained elevation of serum TSH. In this model, TSH release from the pituitary is inhibited by circulating T_4. Therefore, factors which affect the distribution and clearance of TCDD and its induction of UGT might be quantitatively linked to alterations in thyroid hormone levels. Rodents exposed to TCDD develop thyroid tumors (National Toxicology Program, 1982) which may arise from chronic stimulation of the thyroid by elevated TSH (McClain, 1989). This model is a quantitative description of early events which may lead to this end point. Several epidemiological studies (Fingerhut *et al.*, 1991; Manz *et al.*, 1991; Johnson, 1993) indicated increased thyroid tumor risks in humans exposed to TCDD, suggesting that a similar mechanism may operate in humans as well.

Other issues addressed below include uncertainties in the model, the dependence of tissue TCDD concentrations on TCDD binding by serum protein, limitation by cell membrane transport, and the kinetics of TCDD metabolism.

Uncertainties in the Model

The model has been successful in reproducing data from several experiments, including single and multiple doses and oral and subcutaneous routes of administration, and accurately predicting the observed liver TCDD content at doses between 0.1 and 1.0 ng/kg/day. Although the optimal parameter values cannot be shown to be unique, repeated optimizations starting from different initial estimates for these values gave similar results. The selected set of values was the most physiologically reasonable and best reproduced data from experiments that were not used to estimate parameter values. The fit to the data was highly dependent on the specification of the model. Several alternative models were investigated; they include omitting loss of TCDD from the liver due to cytotoxicity, neglecting binding of TCDD to serum protein(s), neglecting TCDD inhibition of Type III deiodinase, assigning regulation of TSH release to the pituitary rather than the hypothalamus. In each case, the alternative model failed to reproduce the observed responses.

One source of uncertainty in the model is that the true value of the fat:blood partition coefficient is unknown. Leung *et al.* (1990) estimated a value of 350 based on the kidney partitioning observed by Gasiewicz *et al.* (1983). Their kidney:blood partition coefficient of 20 would correspond to a fat:blood partition coefficient of 917 according to the regression equation of Fiserova-Bergerova and Diaz (1986). Such a value for the fat:blood partition coefficient would lead to even greater discrepancies between the model's predictions and experimental data. Some uncertainties in the parameter values could be reduced if additional experimental data were available. For example, the amount of induced UGT protein as a function of dose would be helpful in identifying parameter values for UGT synthesis on the mRNA template.

UGT is induced by Ah receptor agonists in many extrahepatic tissues, especially the kidney (Hook *et al.*, 1975). Although most of the T_4 glucuronidation occurs in the liver, inclusion of extrahepatic metabolism would have an effect on the numerical values of some parameters in the model. The amount of Ah receptor in these tissues and changes in this amount consequent to TCDD treatment are unknown. Therefore, it was not possible to assess the contributions of other tissues in this model.

Finally, it must be noted that the values of the parameters in this model are those which best reproduce the responses in the rat. These parameter values may not be appropriate for other species. However, if sufficient data exist to permit estimation of the corresponding parameters in another species, the model with those new parameter values could be used to predict responses in that other species too.

Factors Influencing TCDD Distribution

Although inclusion of capillary spaces in the revised dosimetric model did not produce major alterations in the predicted dose–response for biomarker proteins, the optimal values of the diffusion factors do support the notion that TCDD uptake into tissues is limited by transport across cell membranes. Tissues which have discontinuous (e.g., liver) or fenestrated (e.g., kidney) capillary endothelia have larger values for the transport factor than do tissues with continuous capillary endothelia (e.g., muscle). However, the specific filtration constant for kidney is two orders of magnitude greater than that for muscle (Landis and Pappenheimer, 1963), whereas the kidney transport factor in this model is only six times that for muscle. This suggests that restricted transport of TCDD across the cell membrane differs among tissues and plays a major role in TCDD distribution.

Inclusion of capillary spaces as separate compartments in this dosimetric model did not eliminate the requirement for blood TCDD-binding protein at low doses in order to reproduce observed blood TCDD levels. McKinney *et al.* (1985) showed that the water-soluble TCDD analogue *N*-adipyl-1-amino-3,7,8-trichlorodibenzo-*p*-dioxin binds with high affinity to the serum protein thyroxine binding prealbumin. Their molecular modeling work suggests that TCDD itself should also bind to prealbumin in competition with thyroxine. Albro *et al.* (1978) observed a decrease in serum prealbumin in rats two weeks following a single oral dose of 50 μg TCDD/kg. In addition, exposure to TCDD may result in production of other compounds (e.g., conjugated phenolic metabolites of TCDD) which compete with TCDD for binding to the protein. Such competition would reduce the amount of protein accessible to blood TCDD. The structure of the model and the computed decreased blood protein binding of TCDD with increasing dose are consistent with these observations.

The model of Kohn *et al.* (1993) followed Leung *et al.* (1990) in representing metabolism of TCDD by a pseudo-

first-order rate constant scaled by (body weight)$^{-0.3}$. The revised model replaces this representation by Hill kinetics. The optimal Hill exponent of 1.12 slightly emphasizes the biphasic decrease in liver TCDD concentration following a bolus dose (Abraham *et al.*, 1988). Assuming that clearance of TCDD is dominated by its metabolic rate, the whole-body half life is given by

$$t_{1/2} = \frac{\ln(2) \times K^n}{V \times \text{dose}^n} + \frac{\ln(2)}{V},$$

where K, n, and V are the Hill equation parameters. Thus, rather than remaining constant, the specific rate of elimination ($\ln 2/t_{1/2}$) of TCDD decreases as liver TCDD is depleted.

Whole-body TCDD half-life in humans has been estimated by an exponential decay equation as 10–14.1 years for the Vietnam veterans involved in Operation Ranch Hand (Wolfe *et al.*, 1993). The half-life of TCDD in rats is 24.5 days (determined in adipose tissue by Abraham *et al.*, 1988), so extrapolation of clearance to humans with the above Hill equation may lead to large uncertainties. However, if TCDD metabolism is characterized by Hill kinetics in humans as well as in rodents, humans should exhibit a biphasic decrease in liver TCDD also. Such sigmoidal kinetics may contribute to the long half-life in humans.

TCDD-Related Thyroid Tumors Promoted by UGT Induction

This model reproduces the observed effects of TCDD on blood thyroid hormone concentrations, hepatic UGT activity, and the consequent elevation of serum TSH. The model is consistent with the observation that induction of UGT results in increased glucuronidation and biliary excretion of T_4 (McClain, 1989). Several other compounds that induce UGT—β-naphthoflavone, 3-methylcholanthrene, polychlorinated biphenyls, pregnenolone-16α-carbonitrile—also have been shown (Barter and Klaassen, 1992a; Saito *et al.*, 1991) to lower serum T_4 and raise serum TSH.

Exposure to inducers of isoform 1 of UGT has been observed to result in thyroid tumors (McClain, 1989; Capen, 1992). Sewall *et al.* (1994) found morphological alterations of the thyroid characterized by decreased colloidal follicle size, increased follicular cell size, and hyperplasia in rats given repeated oral doses of TCDD. These morphological changes correlate with serum TSH level and are consistent with the hypothesis that promotion of thyroid tumors is mediated by prolonged stimulation of the thyroid by elevated TSH (Hill *et al.*, 1989; McClain, 1989). The present model provides quantitative support for the hypothesis that induction of UGT is an early event in the generation of thyroid tumors by TCDD in the rat. In the context of chronic exposure, the model shows that changes in UGT concentration could explain the observed changes in circulating thyroid hormones and TSH. As induction of UGT parallels thyroid hormonal status, the induced activity of this enzyme may be predictive of thyroid tumor risks.

APPENDIX[1]

K__TCDD__binding__protein

BodyWeight	$= \text{StartWeight} + \frac{0.219674 \times e^{-0.002859 \times \text{dose}} \times \text{time}}{116.345 + \text{time}}$	
StartWeight	= 0.2373286 kg	
BloodVolume	= 0.024*BodyWeight	(Arterial plus venous but excluding tissue blood)
LiverVolume	= 0.03324 × BodyWeight	(A.M. Tritscher, unpublished results)
WhiteFatVolume	= 0.055 × BodyWeight	(Delp *et al.*, 1990)
BrownFatVolume	= 0.008 × BodyWeight	(Pohjanvirta *et al.*, 1989)
MuscleVolume	= 0.635 × BodyWeight	(Includes skin and tail)
VisceraVolume	= 0.046 × BodyWeight	(Delp *et al.*, 1990)
KidneyVolume	= 0.0085 × BodyWeight	(Delp *et al.*, 1990)
GItractVolume	= 0.029 × BodyWeight	(Delp *et al.*, 1990)
PituitaryVolume	= 0.000022 × BodyWeight	(Piva and Steiner, 1972)
ThyroidVolume	= 0.000052 × BodyWeight	(Average of Piva and Steiner, 1972, and Bastomsky, 1977)
LiverBloodVolume	= 0.11 × LiverVolume	(Altman and Dittmer, 1971)
WhiteFatBloodVolume	= 0.05 × FatVolume	(Altman and Dittmer, 1971)
BrownFatBloodVolume	= 0.05 × FatVolume	(Altman and Dittmer, 1971)
MuscleBloodVolume	= 0.013 × MuscleVolume	(Altman and Dittmer, 1971)
KidneyBloodVolume	= 0.102 × KidneyVolume	(Altman and Dittmer, 1971)

VisceraBloodVolume	= 0.1 × Viscera Volume	(Altman and Dittmer, 1971)
GItractBloodVolume	= 0.029 × GItract Volume	(Altman and Dittmer, 1971)
PituitaryBloodVolume	= 0.093 × Pituitary Volume	(Altman and Dittmer, 1971)
ThyroidBloodVolume	= 0.181 × Thyroid Volume	(Altman and Dittmer, 1971)
dose__interval	= 14 day	
lt	= 0.25 day	(Lag time before induced proteins appear in cell)
ConcBloodE2	= 0.185 nM	
V__Protein	= 300 nmol/liter/day	(Maximal production of blood binding protein for TCDD: determined in this study)
Ki__Protein	= 0.0006 nM	(TCDD inhibition of blood protein production; determined in this study)
Ki__prealbumin production	= 0.9 nM	(Adjustable parameter)
critical__TCDD exposure	= 0.6 nmol	(Determined in this study)

Binding Constants

K__TCDD__binding__protein	1 nM	(Blood binding protein for TCDD; adjusted in this study)
K__Ahr	= 0.27 nM	
K__ER__E2	= 0.13 nM	
K__ER__E2OH	= 1.3 nM	
K__CRP1A2__TCDD	= 30 nM	
Kd__Prealbumin	= 2.86 nM	(Sutherland and Brandon, 1976)
Kd__Albumin__T3	= 8200 nM	(Robbins and Johnson, 1979)
Kd__ALbumin__T4	= 1640 nM	(Sutherland and Brandon, 1976)
K__T3__receptor	= 0.052 nM	(Aprilletti *et al.*, 1987)
K__T4__receptor	= 10.87 nM	(Barnes and DeGroot, 1983 liver; Segal and Ingbar, 1986 other tissues)

Constitutive Expression Parameters

AhRexpression	= 1.4553 nmol/liter/day	
ERexpression	= 0.5471 nmol/liter/day	
CYP1A2expression	= 213.4 nmol/liter/day	
P450Red__expression	= 47.45 nmol/liter/day	
EGFRexpression	= 0.69376 nmol/liter/day	
EndoInducer	= 4×10^{-5} nmol/liter	(As TCDD equivalents; adjusted in this study)
Prealb__expression	= 2979.9 nmol/liter/day	(Calculated to reproduce concentration in blood; Sutherland and Brandon, 1976)
Alb__expression	= 2.578×10^{5} nmol/liter/day	(Calculated to reproduce concentration in blood; Sutherland and Brandon, 1976)
BrownFatT3Rexpress	= 1.705 nmol/liter/day	(Calculated to reproduce concentration of Oppenheimer, 1983)
ViscerаT3Rexpression	= 0.541 nmol/liter/day	(Calculated to reproduce average concentration in rapidly perfused

		tissues of Oppenheimer, 1983)
LiverT3Rexpression	= 1.871 nmol/liter/day	(Calculated to reproduce concentration of Oppenheimer, 1983)
KidneyT3Rexpression	= 2.772 nmol/liter/day	(Calculated to reproduce concentration of Oppenheimer, 1983)
PituitT3Rexpression	= 6.999 nmol/liter/day	(Calculated to reproduce concentration of Oppenheimer, 1983)
BrownFatT4Rexpress	= 679.1 nmol/liter/day	(Assumed similar to brain: Segal and Ingbar, 1986)
ViscerаT4Rexpression	= 303.5 nmol/liter/day	(Calculated to reproduce average concentration in rapidly perfused tissues of Segal and Ingbar, 1986)
LiverT4Rexpression	= 169.8 nmol/liter/day	(Calculated to reproduce concentration of Barnes and DeGroot, 1983)
KidneyT4Rexpression	= 2301 nmol/liter/day	(Calculated to reproduce concentration of Segal and Ingbar, 1986)
PituitT4Rexpression	= 104.0 nmol/liter/day	(Calculated from Segal and Ingbar, 1986)
LiverUGTexpression	= 7.803 nmol/liter/day	(Optimized to fit mRNA data of Vanden Heuvel *et al.*, 1994)
Gene Induction Parameters		
V__AhRinduction	= 8 nmol/liter/day	(Adjusted in this study)
K__AhRinduction	= 4 nM	
V__CYP1A1induction	= 770.5 nmol/liter/day	(Vanden Heuvel *et al.*, 1994)
K__CYP1Ainduction	= 30.38 nM	(Vanden Heuvel *et al.*, 1994)
Ka__Ah	= 18.19 nM	(Vanden Heuvel *et al.*, 1994)
protected__1A1mRNA	= 5.778×10^{-7} nmol/g	(Vanden Heuvel *et al.*, 1994)
V__CYP1A1synthesis	= 3319 nmol/liter/day	
K__CYP1A1synthesis	= 1.151 nM	(Kohn *et al.*, 1994b)
n__CYP1A1synthesis	= 0.5585	(Kohn *et al.*, 1994b)
V__CYP1A2induction	= 4822 nmol/liter/day	
K__CYP1A2induction	= 7.458 nM	
V__ERinduction	= 3.2488 nmol/liter/day	
K__ERinduction	= 0.35 nM	
Ki__Erinduction	= 3.1 nM	
V__TGFinduction	= 1.5 nmol/liter/day	
K__TGFinduction	= 8 nM	
Ka__TGFinduction	= 0.3 nM	
V__UGTinduction	= 489.7 nmol/liter/day	(Optimized to fit data of Vanden Heuvel *et al.*, 1994)
K__UGTinduction	= 46.2 nM	(Optimized to fit data of Vanden Heuvel *et al.*, 1994)
protected__UGTmRNA	=8.9884×10^{-6} nmol/g	(Optimized to fit data of Vanden Heuvel *et al.*, 1994)
V__UGTsynthesis	=370 nmol/liter/day	(Adjustable parameter)
K__UGTsynthesis	=4nM	(Adjustable parameter)
Metabolic Parameters		

V__metabolism	= 5.27 nmol/liter/day	(Determined in this study)
K__metabolism	= 0.67 nM	(Determined in this study)
n__metabolism	= 1.12	(Determined in this study)
V__E2H	= 8496 day^{-1}	
K__E2	= 9400 nM	
K__Reductase	= 83.5 nM	
V__liverDeiodinaseI	= 1.512×10^6 nmol/liter/day	(Leonard and Visser, 1986)
V__kidneyDeiodinaseI	= 1.063×10^6 nmol/liter/day	(Leonard, 1991)
V__thyroidDeiodinaseI	= 9.446×10^5 nmol/liter/day	(Assumed same specific activity as the kidney)
K__DeiodinaseI	= 3620 nM	(Leonard and Visser, 1986)
V__lowKmDeiodinaseI	= 1680 nmol/liter/day	(Chopra, 1991)
K__lowKmDeiodinaseI	= 26 nM	(Chopra, 1991)
V__DeiodinaseII	= 3.61×10^4 nmol/liter/day	(Visser *et al.*, 1983)
K__DeiodinaseII	= 0.9 nM	(Visser *et al.*, 1983)
V__liverDeiodinIII	= 1.814×10^6 nmol/liter/day	(Leonard and Visser, 1986)
V__kidneyDeiodinIII	= 9.331×10^5 nmol/liter/day	(Assumed same specific activity as the liver)
V__visceraDeiodinIII	= 8.294×10^5 nmol/liter/day	(Assumed same specific activity as the liver)
K__DeiodinaseIII	= 60 nM	(Adjustable parameter)
Ki__T4	= 1.3 nM	(Leonard and Visser, 1986)
Ki__DeiodinIII__TCDD	= 2 nM	(Adjustable parameter)
V__UGT	= 21780 day^{-1}	(Coughtrie *et al.*, 1987)
K__UGT	= 350 nM	(Adjustable parameter)

Rate Constants

k__absorption	= 4.8 $kg^{0.75}$/day	
k__proteolysis	= 0.693 day^{-1}	
k__endocytosis	= 0.271 day^{-1}	
k__urine	= 5.36 day^{-1}	(Excretion of TCDD metabolites)
k__bile	= 3.81 day^{-1}	(Excretion of TCDD metabolites)
k__feces	= 1.152 day^{-1}	(Excretion of TCDD metabolites)
k__conjugation	= 56.693 day^{-1}	(Formation of glucuronide derivatives of catechol estrogens)
k__lysis	= 200 day^{-1}	(Specific rate of clearance of TCDD due to hepatocytotoxicity; adjusted in this study)
k__RNA__degradation	= 6.93 day^{-1}	(Kohn *et al.*, 1994b)
k__peptide__degradn	= 332.7 day^{-1}	(Spira and Gordon, 1986)

Partition and Transport Factors for TCDD

FatPartition	= 425	(Adjusted in this study)
Muscle Partition	= 30	(Andersen *et al.*, 1993)
VisceraPartition	= 20	(Andersen *et al.*, 1993)
LiverPartition	= 20	(Andersen *et al.*, 1993)
KidneyPartition	= 20	(Gasiewicz *et al.*, 1983)
GItractPartition	= 20	(Assumed same as for viscera)
FatTransport	= 0.06	(Estimated in this study)
MuscleTransport	= 0.1	(Estimated in this study)
VisceraTransport	= 0.3	(Estimated in this study)

LiverTransport	= 0.6	(Estimated in this study)
KidneyTransport	= 0.6	(Estimated in this study)
GItractTransport	= 0.3	(Estimated in this study)
Partition Coefficients for T3 and T4		(All these partition coefficients were estimated from published K_{ow} values; Pardridge and Mietus, 1980)
FatPartitionT3	= 38.7	
MusclePartitionT3	= 1.707	
GItractPartitionT3	= 1.707	
Viscera PartitionT3	= 1.960	
LiverPartitionT3	= 2.222	
KidneyPartitionT3	= 1.461	
ThyroidPartitionT3	= 1.960	
PituitaryPartitionT3	= 1.960	
FatPartitionT4	= 24.8	
MusclePartitionT4	= 1.301	
GItractPartitionT4	= 1.301	
Viscera PartitionT4	= 1.442	
LiverPartitionT4	= 1.632	
KidneyPartitionT4	= 1.167	
ThyroidPartitionT4	= 1.442	
PituitaryPartitionT4	= 1.442	
T3 and T4 Uptake Parameters		(All of these thyroid hormone uptake parameters were obtained from Krenning *et al.*, 1981)
low__affinity__Km__T3	= 2800 nM	
V__LiverLowAffin__T3	= 4.892×10^8 nmol/liter/day	
V__KidneyLowAffin__T3	= 1.482×10^8 nmol/liter/day	
V__VisceraLowAffin__T3	= 1.028×10^7 nmol/liter/day	
high__affinity__Km__T3	= 0.061 nM	
V__LiverHighAffin__T3	= 5.727×10^6 nmol/liter/day	
V__KidneyHighAffin__T3	= 1.735×10^6 nmol/liter/day	
V__ViscerHighAffini__T3	= 1.203×10^5 nmol/liter/day	
low__affinity__Km__T4	= 1000 nM	
V__LiverLowAffin__T4	= 4.176×10^8 nmol/liter/day	
V__KidneyLowAffin__T4	= 1.264×10^8 nmol/liter/day	
V__VisceraLowAffin__T4	= 8.764×10^6 nmol/liter/day	
high__affinity__Km__T4	= 0.0086 nM	
V__LiverHighAffin__T4	= 1.026×10^6 nmol/liter/day	
V__KidneyHighAffin__T4	= 3.106×10^5 nmol/liter/day	
V__ViscerHighAffin__T4	= 2.150×10^4 nmol/liter/day	
Hormone Relapse Parameter		
V__TRH__release	= 2000 nmol/liter/day	(Adjustable parameter)
Ki__TRH__release	= 4.8 nM	(Gershengorn, 1983)
V__SS__release	= 2211 nmol/liter/day	(Calculated from data of Shoemaker *et al.*, 1983)
Ka__SS__release	= 4.8 nM	(Assumed same as K_i for TRH release)
V__TSH__release	= 1.717×10^6 nmol/liter/day	(Labrie *et al.*, 1978)

Ka__TRH	= 0.3 nM	(Gershengorn, 1983)
Ki__SS	= 0.25 nM	(Labrie *et al.*, 1978; Gershengorn, 1983)
basal__T4__release	= 21450 nmol/liter/day	(Tal *et al.*, 1986)
basal__T3__release	= 1912 nmol/liter/day	(Calculated from T_4:T_3 release ratio of Hotta *et al.*, 1991)
V__T4__release	= 9.266×10^4 nmol/liter/day	(Tajima *et al.*, 1985)
V__T3__release	= 8273 nmol/liter/day	(Calculated from T_4:T_3 release ratio of Hotta *et al.*, 1991; comparable to value of Tajima *et al.*, 1985)
K__TSH	= 0.01 nM	(Adjustable parameter)

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